LARVAL DEVELOPMENT IN *HETERONYMPHA MEROPE MEROPE* (FABRICIUS) (LEPIDOPTERA: NYMPHALIDAE)

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Abstract

In New South Wales, newly hatched larvae of *Heteronympha merope merope* (Fabricius) in autumn are bimodal with respect to feeding behaviour. Non-feeding larvae failed to survive or commence feeding when held in warm (28°C) temperatures and long (15 h) photoperiod (P). Feeding larvae developed rapidly in these conditions to the final instar but were unable to pupate. Post-hatching exposure (1-3 months) of feeding larvae to autumn-winter conditions of cool temperatures and declining photoperiod, prior to transfer to 25°C, 15 h P, enabled development through to adulthood. Larval development in *H. merope* is discussed with respect to the apparent existence of diapause and host grass availability.

Introduction

Heteronympha merope merope (Fabricius), the common brown butterfly, is widespread in southeastern Australia, occurring in a wide range of habitats from metropolitan gardens to mountain forests (Common and Waterhouse 1981). In New South Wales adults first appear in October-November with males exhibiting marked protandry (Edwards 1973). Edwards (1973) suggested that females aestivate and showed that although mating occurred soon after emergence in spring, ovarian development did not occur until autumn, when eggs are laid on soft grass host plants. James (1988) presented additional data confirming aestivation and summer reproductive dormancy in *H. merope*. Females were unable to develop reproductively, regardless of temperature and daylength, until early January, suggesting the existence of an obligatory reproductive diapause. After a period of exposure to summer-like conditions egg production is stimulated by cool temperatures and shortening photoperiods (James 1988).

Oviposition by *H. merope* generally occurs during March-April (Edwards 1973, Fisher 1978), although James (1988) showed that females were gravid as early as mid February in the lower Blue Mountains. Fisher (1978) reported that eggs laid in April hatched after about 12 days. The larvae feed actively but are still 'early instars' in June and July (Fisher 1978).

This paper provides information on the dynamics of larval development and an apparent second diapause in the life cycle of *H. merope*.

Materials and Methods

Initial observations (1984)

Ten to 15 female *H. merope* collected in early March at Hazelbrook in the Blue Mountains, west of Sydney, were allowed to oviposit on potted kikuyu and other soft grasses in a constant growth cabinet (CGC) set at 25°C with a 10 hour photoperiod. The butterflies were confined in a steel-framed, muslin covered cage and fed a 10% sugar/water solution. Eggs were collected,

placed on moistened filter paper in glass petri dishes and held at 28°C under a 10 hour photoperiod. Upon hatching (20 March-6 April), ~100 larvae were transferred with a fine brush to potted grass and held in a temperature controlled glasshouse (range 22-30°C) under naturally declining photophase (12-10 h).

Laboratory studies (1985)
Fifteen to 20 females collected in early April at Hazelbrook were allowed to oviposit during 9-21 April on potted grass in a temperature controlled (range 22-30°C) glasshouse under naturally declining photophase (~10 h). The butterflies were confined in a wooden framed, muslin covered cage and fed a 10% sugar/water solution. Eggs began hatching on 20 April and most of the potted grass and eggs were transferred to a constant temperature room (CTR) (28±1°C/15 h photophase (P)) (summer-like conditions) on 24 April. A small number of eggs (~50) in a single pot of grass were kept outdoors at Hazelbrook from 24 April until 16 August, when larvae were transferred to summer-like conditions until adult emergence. The major group of feeding larvae (~120) was maintained and monitored under the summer-like conditions until 5 July, when remaining individuals (~60) were split equally between (1) a CTR at 28°C/15 h P, (2) a glasshouse at 20-25°C and natural daylength (~10 h) and (3) a CGC at 16-18°C/7 h P. The few larvae remaining at the end of July were combined into a single colony on potted grass and held under ambient laboratory conditions (15-23°C/natural P).

Laboratory studies (1998)
Twenty-four females were collected on 29 March from open woodland adjacent to the Murrumbidgee River at Yanco in southern New South Wales. The following day they were placed with potted kikuyu in a steel-framed, muslin covered cage in a CTR set at 27.5°C/15 h P and fed a 10% sugar/water solution. Three experiments were conducted to determine whether larvae undergo diapause as judged by the duration and success of development under summer-like conditions, preceded or not by varying exposure to ambient autumn/winter temperature/photoperiod.

On 6 April, approximately 40 newly laid eggs were removed from the oviposition cage, placed on moistened filter paper in a glass petri dish and incubated at 5°C (CTR) for four weeks, before transfer to 25°C/15 h P (CTR). Similarly, a group of larvae (n~25) which refused to feed since emergence 14 days earlier, were incubated at 5°C for two weeks before transfer to 20°C/15 h P. Mortality and development of larvae were recorded for both groups.

Two pots of kikuyu grass which recruited approximately 100 eggs each during exposure to female *H. merope* over two days (30-31 March), were held outdoors in wooden/muslin cages under ambient temperature and photoperiod from 1 April to 20 July. Groups of larvae (14-25 individuals)

were collected from the cages on 14 April (~4 days after hatching), 18 May, 15 June and 20 July. They were held in plastic/muslin rearing containers with potted grass in a CTR set at 25±1°C/15 h P. Larvae sampled on 14 April contained feeding and non-feeding individuals whilst subsequent samples contained feeding larvae only. Mortality and development of larvae in the summer-like conditions were recorded.

Results

Initial observations (1984)

Females laid eggs readily, attaching them to grass blades and stalks as well as cage sides and on a few Compositae plants mixed in with the grass. Eggs took 8-9 days to hatch and by 6 April most had hatched. Newly hatched larvae were around 1.5 mm long and creamy white in colour. A distinct bimodalism in feeding behaviour was observed, with ~30% of larvae feeding within hours of emergence and becoming green in colour. The remainder became immobile taking up positions close to the hatched egg and not feeding. These larvae remained creamy-white in colour and died after 2 to 4 weeks. By 15 May, most feeding larvae were in late first or early second instar, 1-1.5 cm long and green. Mortality of larvae increased rapidly during June and the colony died out by early July.

Laboratory Studies (1985)

Newly emerged larvae again showed feeding or non-feeding behaviour. All non-feeding larvae died within 2-3 weeks. The feeding larvae showed good early development under summer-like conditions, with rapid growth and little mortality during the first 4-5 weeks. During weeks 4-9, development slowed and more than 50% of larvae died from moulting difficulties and disease. Survivors reached their final instar by the end of June (9 weeks). All larvae retained in the 28°C environment died by the third week of July. High mortality continued amongst the final instar larvae transferred to the two milder environments and no pupation occurred. Twenty-two survivors subsequently transferred to laboratory conditions died by early September without pupating.

Twelve second and third instar larvae from the outdoor colony transferred to summer-like conditions in mid-August, showed rapid growth and no mortality, reaching the final instar within two weeks. All larvae pupated in early September and emerged as adults at the end of the month.

Laboratory studies (1998)

Newly laid eggs, incubated at 5°C for four weeks, appeared to show embryonic development (internal darkening), but failed to hatch when subsequently held at 25°C/15 h P. Non-feeding first instar larvae, held at 5°C for two weeks, failed to feed and died within a week when subsequently exposed to 20°C/15 h P. A single individual commenced feeding but died after two weeks.

A sample of 50 larvae (four days old) held at 25°C/15 h P, yielded 36 (72%)
non-feeders and 14 (28%) feeders after 14 days. All of the non-feeders died
within three weeks. After four weeks only four feeders remained alive and
these died after eight weeks, after reaching the final instar (Table 1).
Outdoor larvae (second instars), transferred to 25°C/15 h P after one month
(18 May) developed fairly slowly and only 54% reached third instar after
about six weeks (Table 1). The first pupae were formed after nine weeks
with 32% of larvae reaching this stage. The first adult emerged after 13
weeks and the last after 16 weeks, with 22% of larvae reaching adulthood
(Table 1). Larvae transferred to 25°C/15 h P after two months outdoors
(second instars) developed faster and had improved survivorship with 45%
pupating after 7-10 weeks. A total of 27% reached adulthood after 10-12
weeks (Table 1). Outdoor larvae on 20 July (three months since hatching)
were still in the second instar and showed similar development and survival
rates to larvae transferred after two months (Table 1).

Table 1. Development of *Heteronympha merope* at 25°C/15 h P following transfer
of larvae hatched 10-14 April from outdoor conditions at Yanco, NSW during April-
June 1998.

Post-hatch period outdoors	% larval mortality	% pupation (n)	% eclosed adults (n)	Mean (±SE) larval duration (d)
NIL	100 (21)	0	0	-
14.iv - 18.v (34d)	68 (17)	32 (8)	22 (5)	101.6±4
14.iv - 15.vi (62d)	54 (12)	45 (10)	27 (6)	76.7±3
14.iv - 20.vii (88d)	43 (6)	57 (8)	36 (5)	72.2±3

Discussion

H. merope larvae in southern Australia develop very slowly during autumn
and winter, taking 6-7 months to reach pupation (Common and Waterhouse
1981, Fisher 1978). This study suggests that the development of larvae is
regulated by diapause.

First instar larvae occur in two behavioural forms, feeders and non-feeders.
The fate of non-feeding first instars is unknown and should be investigated
(do they die or do they gain feeding competency later in autumn/winter?).
The fact that non-feeding larvae in this study did not survive exposure to, or
begin feeding under summer-like conditions, suggests that they are in
diapause (see below).

Larvae of *H. merope* that feed upon hatching take advantage of the normally
favourable temperature conditions and usually abundant host grass resources
in April-May, and develop to second instars by early winter (Fisher 1978 and
this study). However, second instar larvae in mid-autumn, compared to the

same stage larvae in late autumn-late winter, differ in their competency to complete development under summer-like conditions. Second instar larvae in mid-autumn, exposed to long days and warm temperatures, are unable to pupate, whilst larvae collected during late May-late August pupate successfully under summer-like conditions. Thus, mid-autumn larvae appear to be refractive with respect to full development. A refractory, conditioning or restoration period (defined as a temporary inability to develop under normally favourable environmental stimuli) is a key characteristic of diapause (Danks 1987). If the prolonged larval period/dormancy in *H. merope* was simply quiescence caused by the direct effect of cool temperatures and short daylengths, inhibition of development under favourable conditions would not occur at any time. Similarly, the inability of non-feeding first instar larvae to survive, feed or develop under summer-like conditions, is also indicative of diapause.

Feeding larvae gained competency to complete development after exposure for only one month (14 April-18 May) to ambient conditions. Temperatures were relatively high (mean daily maximum = 21°C, range 15-27°C) at this time suggesting that declining daylength, rather than temperature, facilitated diapause development (Tauber and Tauber 1976). The apparent diapause in *H. merope* larvae does not appear to be instar-specific. Diapause was clearly broken in second and third instar larvae that showed rapid development under summer-like conditions, after collection from outdoors in August 1985. These larvae may have been first instar 'non-feeders' and perhaps completed diapause development in that instar. In contrast, larvae exposed to summer-like conditions from hatching, developed to the final instar before reaching an apparent physiological 'block' to development.

Autumn germination and development of soft grasses, particularly in inland areas of south eastern Australia, is critical to the development and survival of *H. merope* larvae. However, the arrival of autumn 'rains' is sometimes delayed and growth of grasses may not occur until late May/early June in some years. The apparently fixed occurrence of non-feeding first instar larvae of *H. merope,* may be an example of a 'bet-hedging' strategy to ensure that a significant portion of the population survives until host grasses become available. It would be interesting to compare the proportion of non-feeders to feeders in coastal and inland populations of *H. merope*.

A number of insects have been reported to have two or more dormant stages in their life cycle (Danks 1987 and references therein). Larval dormancy and adult aestivation has been recorded in some carabid beetles (Thiele 1969), sciomyzid flies (Berg *et al.* 1982), the lacewing *Nineta flava* (Canard 1982, 1983) and the moth *Triphaena pronuba* (Akhmedov 1977). Dormancies in two stages does not appear to have been reported previously from nymphalid butterflies.

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